THE HORMONAL CONTROL OF METABOLISM IN CRUSTACEANS. IX. CARBOHYDRATE METABOLISM IN THE TRANSITION FROM INTERMOULT TO PREMOULT IN CARCINIDES MAENAS

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Recent reviews of the metabolic events in the intermoult cycle of decapod crustaceans, and of the hormonal control of these events, have emphasized the fragmentary nature of our present knowledge (Knowles and Carlisle, 1956; Scheer, 1957). Particular interest centers around the metabolism of carbohydrate, which is known from the work of Renaud (1949) to undergo considerable changes in the course of the intermoult cycle. The present report is based on a study of a laboratory population of approximately 100 specimens of the crab *Carcinides* (=*Carcinus*) *maenas*, in which the content of total carbohydrate, total soluble polysaccharide, blood carbohydrate, blood lipochromes, and total non-protein nitrogen was determined on the individuals in samples drawn at intervals from the population.

Materials and Methods

The animals were taken from a lagoon north of Banyuls-sur-Mer and brought into the laboratory on October 11; they were maintained throughout the experiment in large aquaria in running sea water, and fed regularly on mussels. Examination of the animals showed them all to be in the hard-shelled condition (stages C_2 through D_2 of Drach, 1939), but closer determination of intermoult cycle stage was not made until the animals were killed for analysis. Most of the animals were males, and only males were used for the studies reported, to avoid complications arising out of sexual differences. On October 14, November 13, and December 1, samples of 20 to 30 crabs, selected at random, were drawn from the group, and the eyestalks were removed from every second animal. Mortality was very low. It is probable that a few animals moulted during the period of the experiment, but cannibalism prevented any certain determination of this.

Eight to ten days after the sampling, the animals were extracted for analysis. The stage in the intermoult cycle was carefully determined, using the criteria of Drach (1939). For this study, an exact determination of the division between the end of the intermoult period (C_4) and the beginning of the premoult period (D_1) was essential. Accordingly, microscopic examination of the external branchial epipodite of the first maxilliped was made to determine the presence of newly formed setae beneath the old integument of this appendage. The presence of even the most rudimentary new setae was taken as an index of the beginning of stage D_1. These rudimentary setae can be detected only by careful microscopic examination under good illumination by transmitted light.

A blood sample was taken by bleeding from a cut walking leg. The animal was then quickly cut up into 50–75 ml. of 5% trichloracetic acid; Renaud (1949) had already shown that the elaborate precautions to prevent glycolysis which are necessary in mammals are not as important in crabs. The mixture of acid and tissue was transferred to an electric blender (Cadillac Atomixer), and blended at 8000 rpm. for 3 minutes. The mixture was rapidly filtered with suction, and the residue returned to the blender with a second portion of acid for a second extraction. The blender and residue were washed with a third portion of acid. The combined filtrates were then diluted in a volumetric flask, usually to 250 ml., and stored in the refrigerator until analyzed, always within a few days.

Blood carbohydrate was determined on some samples by the anthrone method of Roe (1955). One ml. of blood was collected by dripping from a cut walking leg, into a calibrated tube. One ml. of 5% trichloracetic acid was added with mixing, and the mixture was centrifuged. One ml. of the supernatant was then transferred to a second tube for colorimetric determination. Blood lipochromes were determined on other samples. To 1 ml. of blood, 5 ml. of acetone were added. The acetone solution was then extracted with 2 ml. of petroleum ether, the ether layer was washed with water, dried with solid KOH, and diluted to 5 ml. with petroleum ether. The concentration of lipochromes was then read in the spectrophotometer at 450 mμ against a petroleum ether blank. The measurements are given as optical densities, since the exact nature of the lipochromes involved is not known.

Total carbohydrate was determined by the anthrone method (Roe, 1955). One hundred microliters (μl) of the extract were transferred to a tube with a micropipette, and diluted to one ml. for colorimetric determination. Polysaccharide was determined by the same method. To 1 ml. of extract, 5 ml. of 95% ethyl alcohol were added, and the mixture allowed to stand overnight in the refrigerator. The tubes were centrifuged, the precipitate carefully drained, and suspended in 10 ml. of distilled water. A one-ml. sample of this suspension was used for colorimetric analysis. The anthrone method has the advantage for this study that it determines a variety of carbohydrates, and relatively few other naturally occurring compounds. All results are expressed in terms of glucose equivalents.

Non-protein nitrogen (NPN) was determined on 10-ml. samples of the extract, using the micro-Kjeldahl digestion method of Hiller *et al.* (1948) and distilling the digested mixture into 0.1 N HCl in an all-glass still. Ammonia nitrogen (NH_3N) was separated by distilling the undigested extract in the same still. The final determination of ammonia in both cases was colorimetric, using the Nessler reagent.

Results

In the first sample, examined 11 to 19 days after collection, 10 out of 16 animals (63%) were in the C_4 stage (late intermoult) of the intermoult cycle; the remainder were in the D_1 stage (early premoult). In the second sample, examined 43 to 45 days after collection, the proportion of C_4 animals was 52% (12 C_4, 8 D_1, 3 D_2). In the third sample, the proportion was 32% (7 C_4, 15 D_1). These values suggest that the population from which the samples were drawn was undergoing a steady progression towards the moult. The χ^2 test shows that the proportion of C_4 animals in the third sample is significantly less than in the first, at the 5% level of probability.

In the first sample, only one of the 10 C_4 animals had blood clearly pigmented with lipochromes, while 5 of the 16 D_1 animals had blood so pigmented; no quantitative determinations were made in this series. In the second sample, 5 of the 12 C_4 animals and 6 of the 11 D_1 animals had lipochromes clearly evident in the blood; quantitative measurements were made on these 11 animals, and are presented in Table I. For the third sample, quantitative measurements were made on all the animals, and are presented in Table I. From the results on the third sample, in which traces of lipochrome are found in nearly all specimens, it appears that the level for qualitative detection of lipochromes lies at about 0.05 on the density scale used to express concentrations. On this basis, we would conclude that only one of the 7 C_4 animals of the third sample had substantial amounts of lipochrome in the blood, while 7 of the 15 D_1 animals had such amounts. If we apply the χ^2 test to

TABLE I

Lipochromes in the blood of Carcinides maenas. Optical density at 450 mμ of a petroleum ether extract, volume 5 ml., from 1 ml. of blood. The values for sample 2 (see text) represent only animals in which blood lipochromes were qualitatively evident

Stage condition	C_4				D_1			
	Normal		Eyestalkless		Normal		Eyestalkless	
	Animal	Density	Animal	Density	Animal	Density	Animal	Density
Sample 2	22	0.072	24	0.064	31	0.150	35	0.070
	36	0.065	29	0.059	34	0.077	23*	0.157
			33	0.112	40	0.088		
					42*	0.076		
Sample 3	45	0.063	44	0.016	47	0.000	48	0.000
	49	0.010	46	0.020	55	0.035	50	0.055
	51	0.015	54	0.010	57	0.030	52	0.020
	53	0.012			59	0.026	56	0.050
					61	0.105	58	0.090
					63	0.095	60	0.010
					65	0.066	62	0.210

* Stage D_2.

these values, we find that the frequency of occurrence of easily observable amounts of lipochrome in the blood is not significantly different from 1 in 10 animals for the C_4 stage in samples 1 and 3, but is significantly different, at the 10% level of probability or better, for all the other groups. The 1:10 ratio observed in C_4, sample 1, is also significantly different from the 1:2 ratio observed in D_1, sample 3.

The mobilization of lipochromes from the digestive gland to the integumentary tissues is an important part of the preparation for the moult, and all of the D_1 animals in this study showed deposits of pigment in the region of the membranous layer of the integument; indeed, this characteristic appears to be a fairly reliable means of detecting the beginning of the premoult period. The appearance of lipochrome in substantial amounts in the blood may therefore be taken as an indication of the beginning of preparations for moulting. It is clear from the results presented that this mobilization begins before the first morphological signs of premoult (initia-

tion of new setae) appear. Moreover, we may conclude that the C_4 animals in the second sample were further advanced towards the premoult stage than were those in the first or third samples. There is no conclusive evidence that eyestalk removal has any effect on the mobilization of lipochromes.

The results of the carbohydrate determinations are presented in Table II. We may first note the rather striking difference in carbohydrate content of normal animals in stage C_1 between sample 2 and the other two samples. The mean values

TABLE II

Total carbohydrate and polysaccharide content (mg. glucose equivalent per gm. body weight) of three samples from a population of Carcinides maenas, and the effect of eyestalk removal

Stage condition	C_4						D_1					
	Normal			Eyestalkless			Normal			Eyestalkless		
	No.	Carb.	Poly-sac.	No.	Carb.	Poly-sac.	No.	Carb.	Poly-sac.	No.	Carb.	Poly-sac.
Sample 1	7	2.14	1.31	6	5.64	4.04	11	3.63	3.00	13	3.34	2.06
11–19 days	8	0.59	0.24	9	9.50	7.48	19	2.93	1.85	18	6.48	5.42
	12	2.38	1.70	10	8.59	7.67	20	6.10	5.24	21	6.19	5.05
	15	2.18	1.12	14	13.5	11.0						
	16	3.03	1.96	17	4.52	4.01						
Sample 2	22	8.88	8.48	24	9.45	9.20	31	4.83	4.83	35	15.7	13.9
43–45 days	25	12.7	8.57	27	9.53	9.53	32	16.4	16.4	41	5.45	5.25
	26	1.13	1.13	29	7.40	7.32	34	8.84	8.52	43	14.4	12.8
	28	15.7	14.8	30	12.8	12.3	40	14.4	13.7	23*	15.0	15.0
	36	12.2	11.6	33	16.5	15.8	38*	14.8	14.2	42*	23.6	10.9
				37	3.18	3.18						
				39	19.3	16.8						
Sample 3	45	1.36	1.36	44	8.13	8.13	47	1.45	1.45	48	1.72	1.41
67–70 days	49	2.73	2.21	46	9.40	7.06	55	9.41	8.48	50	3.52	2.98
	51	4.10	4.10	54	6.11	5.03	57	16.0	15.2	52	4.90	4.90
	53	2.28	1.88				59	12.8	11.1	56	12.7	11.0
							61	24.4	21.6	58	11.4	11.4
							63	15.7	11.9	60	2.49	1.95
							65	10.7	9.53	62	10.6	8.33
										64	18.9	16.3

* Stage D_2.

for samples 1 and 3 are 2.06 and 2.62 mg. per gm. for total carbohydrate, while the corresponding mean for sample 2 is 10.12 mg. per gm. The difference between the means for sample 1 and 2 is significant at the 5% level on the basis of the *t* test. This difference in means arises from the fact that all but one of the values from sample 2 are greater than 8 mg. per gm., while none of the values from samples 1 and 3 is as great as 5 mg. per gm. Moreover, the single low value in sample 2 was obtained from one of the animals (no. 26) which had no obvious lipochrome in the blood. If our earlier conclusion, that a substantial fraction of the animals in stage C_4 of the second sample were well on their way toward stage D_1, is correct, then we can further conclude that one characteristic of this transition is a marked increase in

the carbohydrate content of the body. This conclusion is confirmed by the values for normal animals in stage D_1, which are nearly all well above those for the C_4 animals of groups 1 and 3. The difference between mean values for sample 3 for C_4 and D_1 is significant at the 1% level on the basis of the *t* test. Renaud (1949) had already observed a similar change in *Cancer pagurus* with a mean glycogen content of 2.09 mg. per gm. for animals in C_4, rising to 4.43 mg. per gm. by the end of D_1. We may therefore conclude that the increase in carbohydrate content which is characteristic of the transition from intermoult to premoult may occur during the latter part of stage C_4, before any morphological evidence of the transition is apparent.

TABLE III

Non-protein nitrogen (NPN) and ammonia nitrogen (NH_3N) in normal and eyestalkless Carcinides maenas (mg. per gm. body weight)

Stage condition	C_4						D_1					
	Normal			Eyestalkless			Normal			Eyestalkless		
	No.	NPN	NH_3N	No.	NPN	NH_3N	No.	NPN	NH_3N	No.	NPN	NH_3N
Sample 1	8	2.40	0.25	6	2.45	0.10	11	2.44	0.13	10	2.56	0.10
	12	2.89	0.17	9	2.52	0.08	19	2.84	0.05	13	3.07	0.12
	15	2.88	0.20	14	3.58	0.08	20	3.39	0.06	18	3.08	0.05
	16	2.58	0.07	17	2.65	0.08				21	3.82	0.04
Sample 2	22	2.90	0.16	24	2.68	0.24	31	2.91	0.19	35	2.96	0.30
	25	3.21	0.20	27	3.96	0.24	32	3.97	0.28	41	3.17	0.29
	26	2.03	0.13	29	3.22	0.23	34	3.13	0.24	43	3.30	0.25
	28	4.74	0.23	30	3.51	0.26	40	3.48	0.28	23*	3.17	0.32
	36	2.82	0.25	33	4.07	0.27	42*	3.69	0.42			
				37	2.72	0.19	38*	3.03	0.26			
				39	2.64	0.27						

* Stage D_2.

The second item to be noted from Table II is the fact that the carbohydrate content of the eyestalkless animals in C_4 is throughout at levels characteristic of D_1 animals. Indeed, there was no eyestalkless C_4 animal with a carbohydrate content as low as 3 mg. per gm., and in all but two, the value was higher than 5 mg. per gm. The differences in means for normal and eyestalkless animals in C_4 were significant at the 5% level for both samples 1 and 3, on the basis of the *t* test. We may therefore conclude that the operation of eyestalk removal causes an increase in carbohydrate content from the low values characteristic of C_4 animals to the higher values characteristic of the next stage in the cycle, D_1. The same operation is clearly without effect upon animals already in stage D_1 if for some reason these animals have low carbohydrate content, since there are several eyestalkless D_1 animals with relatively low carbohydrate values, and the distribution of values in normal and eyestalkless specimens in this stage is substantially the same. We may further infer from our results, though conclusive evidence is lacking, that some endocrine factor is secreted in the eyestalk during stage C_4, and that secretion of this factor stops towards the end of that stage. One effect of this factor would be the maintenance

of carbohydrate content at relatively low levels. Since Renaud (1949) has shown a steady increase in glycogen content beginning in stage C_3, we may suppose that the secretion of the factor concerned decreases gradually rather than suddenly.

In general, the polysaccharide values follow the carbohydrate values rather closely, and 80% or more of the carbohydrate is precipitated by alcohol. However, in the C_4 animals of the first sample, the polysaccharide averages only 62% of the total carbohydrate; the eyestalkless individuals, and indeed all of the other groups, had a higher ratio. Blood carbohydrate was measured for the animals of sample 1 only. The results are presented in Table III. Since it appears that the carbohydrate content of the blood does not reflect changes in the total carbohydrate of the body, and is not influenced by any of the other factors considered here, we utilized the blood samples from the second and third group for lipochrome studies.

The observation of Needham (1955) that increased nitrogen excretion follows eyestalk amputation, led us to examine the nitrogen content of some of the extracts. The results are presented in Table IV. There appears to be no systematic variation in either NPN or NH_3N, except that both sets of values, and especially the NH_3N values, are generally lower in the animals of sample 1 than in those of sample 2. No obvious explanation for this difference appears. In both samples, the extracts were prepared 7 to 14 days after eyestalk removal, by which time Needham (1955) found that nitrogen excretion had returned to normal levels. We conclude that no long-lasting modification in nitrogen metabolism evident from NPN or NH_3N content of the animals is related to the variables considered here.

Discussion

Perhaps the most important finding of this study is that metabolic changes (mobilization of lipochromes, increased carbohydrate content) preparatory to the moult precede in time the morphological changes (formation of new setae). This may not be surprising, but it has not been emphasized before. We cannot on the basis of the evidence available conclude that the metabolic changes are causally related to the subsequent structural changes, but this is a reasonable inference. However, the two metabolic changes observed do not seem to be directly related one to the other. There is in general no complete correlation between increased blood lipochrome and increased carbohydrate. Moreover, the increase in carbohydrate which follows eyestalk removal is not in general associated with increased blood lipochrome.

The increase in carbohydrate content as the animal approaches a moult was already known from the study of Renaud (1949) on *Cancer pagurus*. Moreover, Schwabe *et al.* (1952) had observed a marked increase in total glycogen, represented by deposition in the digestive gland and epidermis, in the transition to the premoult stage in spiny lobsters; their data also suggest that eyestalk removal in stage C increases the total glycogen of the body, while the same operation in stage D results in no change. However, they did not determine this quantity directly, and neither of their methods, for determination of glycogen or for determining intermoult cycle stage, was entirely satisfactory. The demonstration of an increased carbohydrate content following eyestalk removal therefore comes as a definite addition to the long list of metabolic and other changes which are consequences of this operation (Knowles and Carlisle, 1956; Scheer, 1957).

The absence of any changes in blood carbohydrate was something of a surprise.

Renaud (1949) found a steady increase in the reducing power of the blood from C_3 through D_1 in *Cancer pagurus*, but this increase was not evident when the blood was treated with cadmium sulfate and sodium hydroxide, a procedure supposed to eliminate non-glucose reducing substances. Recent studies in my laboratory by McWhinnie (unpublished) on the blood of *Hemigrapsus nudus* have shown that the blood carbohydrate, like the total carbohydrate in acid extracts of the body, includes several components, of which glucose is a relatively minor one. Using the highly specific hexokinase glucose-6-phosphate dehydrogenase method, she found glucose concentrations averaging below 2 mg. per 100 ml., with a maximum about 2.5 mg% in stage C_1, and a slight decrease in stage C_3, but no change as a result of eyestalk extirpation. We had earlier found a decrease in the reducing substances of spiny lobster blood (Scheer and Scheer, 1951) following eyestalk removal, but others (Abramowitz *et al.*, 1944; Kleinholz and Little, 1949) found no such change in crabs. It is clear that the problem of blood sugar regulation in crustaceans requires further careful study with particular attention to specificity of methods.

The question now arises, what is the source of the increased carbohydrate in late intermoult, and what alterations in metabolism are responsible for the increase. Related to this is the question of the endocrine factors which we presume to be responsible for the increase. The evidence on which we can base hypotheses remains fragmentary. Injection of eyestalk extracts increases blood reducing substances, and specifically the fermentable reducing substances (Abramowitz *et al.*, 1944; Kleinholz and Little, 1949; Scheer and Scheer, 1951). It would be unwise at present to equate fermentable reducing substances with glucose, and we do not know the metabolic relations among the various carbohydrates found in the blood, nor indeed the identity of these substances. Scheer and Scheer (1951) showed that injected glucose was removed from the blood more rapidly in eyestalkless than in normal spiny lobsters, and that most of the carbon of this glucose could be recovered in the water- and alcohol-soluble fraction of tissue extracts. From the work of Hu (1958) we know that this fraction may contain, besides glucose, several oligosaccharides of the maltose series. But the relation of these substances to synthesis of polysaccharides or other aspects of carbohydrate metabolism remains obscure. Present evidence, from plants and animals alike, indicates that glycoside linkages in general are formed by adding one monosaccharide unit at a time to existing nuclei by the agency of nucleotide coenzymes. Hu (1958) has shown that nucleotides are present in crabs, and that carbon from administered glucose appears in these compounds.

Whatever the intermediate steps, the increased carbohydrate content of late intermoult crabs may be derived ultimately either from protein or carbohydrate or both. The evidence that, in fed crabs, there is no change in the non-protein nitrogen, suggests that there is no fundamental alteration in the intensity of protein metabolism. On the other hand, the evidence of Neiland and Scheer (1953) that, in fasting crabs, protein is used in preference to carbohydrate, and in eyestalkless crabs, the amount of protein used is greater, together with the evidence of Needham (1955) that under conditions (trauma) in which protein breakdown is increased eyestalk removal leads to a further increase, suggest that, in eyestalkless animals there may be an increased conversion of protein to carbohydrate. The fact that, in such animals, the utilization of glucose is also increased (Scheer and Scheer, 1951)

would lead one to place the site of the presumed endocrine effect in the process of glycogenesis, rather than in that of gluconeogenesis. We may, therefore, postulate that, during the C_4 stage, there is a gradual transition from carbohydrate oxidation to polysaccharide synthesis as a major pathway of carbohydrate metabolism. This offers a possible explanation for the difference in glucose oxidation observed by Hu (1958) and Scheer and Scheer (1951) using similar procedures with different animals. The crabs used by Hu may have been in the early part of stage C_4 or even in C_3, when carbohydrate oxidation is dominant; the spiny lobsters used by Scheer and Scheer (1951) may have entered into the phase in which carbohydrate synthesis predominates. Neither author determined the intermoult stage with great accuracy. Or, if we accept the view of Carlisle (Knowles and Carlisle, 1956) that the intermoult cycle is qualitatively different in animals with prolonged intermoults (diecdysis, as in crabs in winter) from the intermoult in animals which moult regularly throughout the year (anecdysis, as in Hawaiian spiny lobsters), we might suppose that carbohydrate oxidation is primarily characteristic of diecdysis and that in anecdysis polysaccharide synthesis predominates. These suggestions can be tested by careful comparison of the fate of carbon from administered labeled glucose in the various stages of moult cycles of both types.

The question of hormonal control is likewise a difficult one. Carlisle (Knowles and Carlisle, 1956) has summarized evidence suggesting that two separate hormones are concerned in the control of the intermoult period. Carlisle attributes diecdysis to the action of the well-known moult-inhibiting hormone, and cites his own observations and some of ours (Scheer and Scheer, 1954) to the effect that this hormone is not active in certain crustaceans, including British populations of *Carcinides maenas* in the summer. We have no way of knowing whether the animals studied in the present investigation undergo a cycle of the type characterized by anecdysis, or one characterized by diecdysis. It would appear that, to make much further progress with these problems, it will be essential to work with at least partly purified hormone preparations, and to have full information about the type of cycle and stage of the animals in the cycle. We earlier postulated (Scheer and Scheer, 1951) an eyestalk factor which restrains carbohydrate utilization for polysaccharide, and specifically chitin, synthesis. This factor would be the same as the "diabetogenic" factor of Abramowitz *et al.* (1944), and we suggested that it might also be the moult-inhibiting factor. The results reported here do not give us any reason to alter this hypothesis, nor do they substantially strengthen it. However, at present it seems best to consider that the effects of eyestalk removal noted by Neiland and Scheer (1953) on protein catabolism, and the related effect noted by Needham (1955), might result from the action of this same factor. For conclusive evidence concerning this hypothesis, it is essential to have a hormone preparation of reasonable purity which can be tested for its specific metabolic effects.

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Summary

1. A laboratory population of *Carcinides maenas* was sampled three times over a period of 70 days, and the blood carbohydrate, blood lipochromes, total body carbohydrate, total body polysaccharide, non-protein nitrogen, and ammonia nitrogen were determined; the effect of eyestalk removal on these quantities was also examined.

2. During the course of the observations, there was a progression within the population from late intermoult (C_4) to early premoult (D) stages.

3. The change from intermoult to premoult was signalized by the appearance of relatively large amounts of lipochromes in the blood and integument, and by an increase in total body carbohydrate content. These biochemical changes preceded any morphological signs of preparation for moult.

4. Eyestalk extirpation caused an increase in the body carbohydrate, but did not alter the blood lipochromes. The increase in carbohydrate was observed only in those animals which had not undergone the change spontaneously.

5. The other quantities measured showed no variation attributable either to the stage in the intermoult cycle or to eyestalk removal.

6. The results are discussed with relation to the possible mechanisms of the effects observed, and the hormonal factors concerned.

LITERATURE CITED

Abramowitz, A. A., F. L. Hisaw and D. N. Papandrea, 1944. The occurrence of a diabetogenic factor in the eyestalks of crustaceans. *Biol. Bull.*, **86**: 1–5.

Drach, P., 1939. Mue et cycle d'intermue chez les crustaces decapodes. *Ann. Inst. Oceanogr. Paris*, **19**: 103–391.

Hiller, A., J. Plazin and D. D. Van Slyke, 1948. A study of conditions for Kjeldahl determination of nitrogen in proteins. *J. Biol. Chem.*, **176**: 1401–1420.

Hu, A. S. L., 1958. *Arch. Biochem. Biophys.* (in press).

Kleinholz, L. H., and B. C. Little, 1949. Studies in the regulation of blood sugar concentrations in crustaceans I. Normal values and hyperglycemia in *Libinia emarginata*. *Biol. Bull.*, **96**: 218–227.

Knowles, F. G. W., and D. B. Carlisle, 1956. Endocrine control in the Crustacea. *Biol. Rev.*, **31**: 396–473.

Needham, A. E., 1955. Nitrogen-excretion in *Carcinides maenas* (Pennant) during the early stages of regeneration. *J. Embryol. Exp. Morphol.*, **3**: 189–212.

Neiland, K. A., and B. T. Scheer, 1953. The influence of fasting and sinus gland removal on body composition of *Hemigrapsus nudus*. Part V of the hormonal regulation of metabolism in crustaceans. *Physiol. Comp. Oecol.*, **4**: 321–326.

Renaud, L., 1949. Le cycle des reserves organiques chez les crustaces decapodes. *Ann. Inst. Oceanogr. Paris*, **24**: 259–357.

Roe, J. H., 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, **212**: 335–343.

Scheer, B. T., 1957. Recent Advances in Invertebrate Physiology, pp. 213–227. Univ. of Oregon, Eugene.

Scheer, B. T., and M. A. R. Scheer, 1951. Blood sugar in spiny lobsters. Part I of the hormonal regulation of metabolism in crustaceans. *Physiol. Comp. Oecol.*, **2**: 198–209.

Scheer, B. T., and M. A. R. Scheer, 1954. The hormonal control of metabolism in crustaceans VII. Moulting and colour change in the prawn *Leander serratus*. *Pubbl. Staz. Zool. Napoli*, **25**: 397–418.

Schwabe, C. W., B. T. Scheer and M. A. R. Scheer, 1952. The molt cycle in *Panulirus japonicus*. Part II of the hormonal regulation of metabolism in crustaceans. *Physiol. Comp. Oecol.*, **2**: 310–320.